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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/52, 14/47, A61K 38/16, 38/17, 38/19, C12N 15/19 — **A1**

(11) International Publication Number: WO 97/15595

(43) International Publication Date: 1 May 1997 (01.05.97)

(21) International Application Number: PCT/US96/17074

(22) International Filing Date: 24 October 1996 (24.10.96)

(30) Priority Data:
08/547,262 24 October 1995 (24.10.95) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/547,262 (CIP)
Filed on 24 October 1995 (24.10.95)

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(81) Designated States: AL, AM, AU, BB, BG, BR, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KG, KP, KR, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD OF MOBILIZING HEMATOPOIETIC STEM CELLS

(57) Abstract

A method of mobilizing hematopoietic stem cells from the bone marrow to the peripheral circulation is provided by administering to an animal an effective amount of nature, modified or multimeric forms of KC, *groβ*, *groα* or *groγ*.

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METHOD OF MOBILIZING HEMATOPOIETIC STEM CELLS

Field of the Invention

The present invention relates generally to methods for mobilizing hematopoietic stem cells.

Background of the Invention

All the members of the intercrine or chemokine family are basic heparin-binding polypeptides which have four cysteine residues which form two disulfide bridges. All these proteins which have been functionally characterized appear to be involved in proinflammatory and/or restorative functions.

In clinical situations for the use of high dose chemotherapy, the biomolecule of choice has been G-CSF. Generally, in such treatment, patients are primed with a low dose of a chemotherapeutic agent like cyclophosphamide. During the remission, the patient is treated with a CSF, such as G-CSF, which causes eventual mobilization of cells from the bone marrow to the peripheral circulation for harvesting of leukophoresed blood. The patient is thereafter administered a high dose of chemotherapy to induce clinical remission of their cancer. The resultant bone marrow failure is treated by infusion of the stored blood cells collected previously. This procedure may be modified, e.g., by the omission of the initial dose of chemotherapy and/or alternate blood collection protocols.

While the use of these hematopoietic stem cell transplantation techniques looks promising, multiple apheresis procedures are required to harvest sufficient stem cells for successful engraftment to treat severe

myelosuppression when G-CSF is used alone [see, e.g., Bensinger *et al*, Blood, 81:3158 (1993) and R. Haas *et al*, Sem. in Oncology, 21:19 (1994)]. Thus, despite these significant advances and the availability of certain regulatory biomolecules, delayed recovery of hematopoiesis remains an important source of morbidity and mortality for myelosuppressed patients.

There exists a continuing need in the art for compositions and methods to enhance hematopoietic recovery, particularly in cases of chemotherapy associated myelosuppression.

Summary of the Invention

In one aspect, the present invention provides for the use of a chemokine in the preparation of a medicament for the stimulation of hematopoietic stem cells. This chemokine includes proteins derived from KC, *groß*, *groα*, and *groγ*, including mature, modified, and multimeric forms of these chemokines.

In yet a further aspect, the present invention provides a method for mobilizing hematopoietic stem cells in an animal comprising administering to an animal an effective amount of a mature or modified or multimeric chemokine as described herein.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

Brief Description of the Drawings

Fig. 1 is a graph demonstrating the effect of *groß* (amino acids 1-73 of SEQ ID NO: 3) in the single agent mobilization assay of Example 1.

Fig. 2 is a graph demonstrating the effect of modified *groß* (amino acids 5-73 of SEQ ID NO: 3) in the single agent mobilization assay of Example 1.

Fig. 3 is a bar graph demonstrating the comparison of phosphate buffered saline (PBS), IL-8, *groß* (amino acids 1-73; SEQ ID NO:3) and modified *groß* (amino acids 5-73 of SEQ ID NO:3) in the single agent mobilization assay.

Detailed Description of the Invention

The present invention provides modified proteins, specifically chemokines, associated with inflammatory responses, hematopoiesis and myelopoiesis, which modified proteins are characterized by having enhanced biological activity as compared to the corresponding unmodified or untruncated mature proteins. The present invention provides methods for the treatment of myelosuppression, by mobilizing hematopoietic stem cells from the bone marrow into the peripheral blood using the mature or modified or multimeric chemokines described herein.

I. Definitions

As defined herein, "hematopoietic synergistic factor" or "HSF" refers to a class of proteins, including the naturally occurring chemokines and modified chemokines, which are characterized by having synergistic activity in stimulating hematopoiesis when administered *in vivo* and *in vitro* with another hematopoietic factor, such as a colony stimulating factor, or combined with naturally circulating CSFs.

The term "mature chemokines" also known as "intercrines", as used herein defines the proteins conventionally referred to in the art as KC, *groα*, *groß*, and *groγ*. For convenience, the amino acid sequences of the murine protein KC which contains 72 residues is

provided in SEQ ID NO:1. These sequences are available from Genbank, accession number J04596. The sequences of the human protein *groα* (aa 1-73) are provided in SEQ ID NO:2. The sequences of the human protein *groß* (amino acids 1-73) are provided in SEQ ID NO: 3. The sequences of the human protein *groγ* are provided in SEQ ID NO:4. The cDNA and amino acid sequences of *groγ* are also provided in International Patent Application, Publication No. WO 92/00326 (Jan. 9, 1992). These *groγ* sequences have further been published in International Patent Application, Publication No. WO 94/29341 (December 22, 1994), which is incorporated by reference herein.

The term "modified chemokines" is defined as in the above-referenced International Application. The modified chemokines are derived from KC, *groß*, *groα*, and *groγ*, more preferably from *groß*, *groα*, and *groγ*, and most preferably from *groß*. The modified chemokines include desamino proteins characterized by the elimination of between about 2 to about 8 amino acids at the amino terminus of the mature protein. These desamino chemokines useful in the method of the invention are preferably characterized by removal of about 2 to about 8 amino acids from the amino terminus of the mature protein. Most preferably, the modified chemokines are characterized by removal of the first 4 amino acids at the amino- (N-) terminus. Optionally, particularly when expressed recombinantly, the desamino chemokines useful in this invention may contain an inserted N-terminal Met. The N-terminal methionine which is inserted into the protein for expression purposes, may be cleaved, either during the processing of the protein by a host cell or synthetically, using known techniques. Alternatively, if so desired, this amino acid may be cleaved through enzyme digestion or other known means.

Also included by the term modified chemokine are analogs or derivatives of these proteins which share the biological activity of the mature protein. As defined herein, such analogs and derivatives include modified proteins also characterized by alterations made in the known amino sequence of the proteins, e.g., the proteins provided in SEQ ID NOS: 1-4. Such analogs are characterized by having an amino acid sequence differing from that of the mature protein by 8 or fewer amino acid residues, and preferably by about 5 or fewer residues. It may be preferred that any differences in the amino acid sequences of the proteins involve only conservative amino acid substitutions. Conservative amino acid substitutions occur when an amino acid has substantially the same charge as the amino acid for which it is substituted and the substitution has no significant effect on the local conformation of the protein or its biological activity. Alternatively, changes such as the introduction of a certain amino acid in the sequence which may alter the stability of the protein, or permit it to be expressed in a desired host cell may be preferred. Another characteristic of these modified proteins may be enhanced biological activity in comparison to the mature protein.

By the term "multimeric protein" or "multimer" is meant herein multimeric forms of the mature and/or modified proteins useful in this invention, e.g., dimers, trimers, tetramers and other aggregated forms. Such multimeric forms can be prepared by synthesis or recombinant expression and can contain chemokines produced by a combination of synthetic and recombinant techniques as detailed below. Multimers may form naturally upon expression or may be constructed into such

multipl forms. Multimeric chemokines may include multimers of the same modified chemokine. Another multimer may be formed by the aggregation of different modified proteins. Still another multimer is formed by the aggregation of a modified chemokine of this invention and a known, mature chemokine. Preferably, a dimer or multimer useful in the invention would contain at least one desamino chemokine protein and at least one other chemokine or other protein characterized by having the same type of biological activity. This other protein may be an additional desamino chemokine, or another known protein.

II. Proteins Useful in the Invention

In general, the chemokines useful in the method of the invention include the mature chemokines, or the modified and multimeric proteins derived therefrom, which are described in detail in International Patent Application, Publication No. WO94/29341. Desirably, these chemokines are selected from KC, *groα*, *groβ* and *groγ*, and most preferably the chemokine is *groβ*.

In one preferred embodiment, the method of the invention utilizes a desamino chemokine protein of the invention. This protein comprises the amino acid sequence of mature chemokine useful in the invention truncated at its N terminus between amino acid positions 2 and 8 of SEQ ID NOS:1-4. Preferably, the desamino protein of the invention has a protein sequence spanning amino acids 5 to 73 of SEQ ID NOS: 2-4, or amino acids 5 to 72 of SEQ ID NO:1. Most preferably, the method of the invention is desamino *groβ*, which has the protein sequence spanning amino acids 5 to 73 of SEQ ID NO:3.

This desamino-*groβ* is characterized by having at least about two logs higher biological activity than unmodified, human *groβ*, as determined in the above-references HSF assay.

As described in WO94/29341, similar modifications can be made to the KC, *groα* and *groγ* proteins which are useful in the methods of the invention. These proteins are all described in the literature and are known to those of skill in the art.

Preferred multimeric proteins useful in this invention include, dimers or multimers containing at least one desamino chemokine protein and at least one other chemokine or other protein characterized by having the same type of biological activity. This other protein may be an additional desamino chemokine, or another, known protein. For example, a desirable dimer useful in the methods of the invention comprises two desamino proteins as described above, preferably linked by disulfide bonds. A desirable multimer may be an aggregate of two or more desamino *groβ* proteins, particularly two proteins consisting of amino acids 5-73 of SEQ ID NO:3. Alternatively, another dimer of the invention may be a desamino *groβ* protein of the invention in combination with a mature *groβ* protein. Similarly, various combinations of dimers or other multimeric forms may contain a combination of the mature or modified *groβ* and other chemokines, such as the KC, *groα* and *groγ* proteins. For example, a desamino *groβ* protein of the invention may form a dimer with an unmodified mature *groα* protein. One of skill in the art may obtain other desirable multimers using the modified chemokines of the invention. However, the use of multimeric forms of two or more different modified proteins as defined herein are

useful in the method of this invention. The chemokine employed in this method may also be a multimeric form of a modified chemokine as discussed above and another known mature protein.

These proteins and monomers have been described in detail in the literature and may be synthesized, or produced recombinantly, using conventional techniques and/or the techniques described in International Patent, Publication No. WO94/29341.

III. Pharmaceutical Compositions

Desirably, the chemokines useful in the method of the invention are used in the preparation of medicaments and/or are useful in the form of a pharmaceutical composition. Thus, the chemokines can be formulated into pharmaceutical compositions and administered in the same manner as described in, e.g., International Patent Applications, Publication No. WO 90/02762 (Mar. 22, 1990) and Publication No. WO94/29341 (Dec. 22, 1994).

These medicaments or pharmaceutical compositions useful in the mobilization of hematopoietic stem cells contain a therapeutically effective amount of a mature, modified or multimeric chemokine as defined herein and an acceptable pharmaceutical carrier. As used herein, the term "pharmaceutical" includes veterinary applications of the invention.

The term "therapeutically effective amount" refers to that amount of a chemokine, whether in monomeric or multimeric form, which is useful for mobilizing stem cells in sufficient amounts to achieve the desired physiological effect.

Generally, a matur , modified or desamino chemokine useful in the invention (e.g., *groβ*) is administered in an amount between about 0.01 ng/kg body weight to about 1 g/kg body weight and preferably about 0.01 ng/kg body weight to 10 mg/kg body weight per dose. Desirably, when a multimeric chemokine is used in the method of the invention, the medicament or composition contains amounts of the multimeric protein at the lower end of this range. Preferably, these pharmaceutical compositions are administered to human or other mammalian subjects by injection. However, administration may be by any appropriate internal route, and may be repeated as needed, e.g. one to three times daily for between 1 day to about one week.

Suitable pharmaceutical carriers are well known to those of skill in the art and may be readily selected. Currently, the preferred carrier is saline. Optionally, the pharmaceutical assays of the invention may contain other active ingredients or be administered in conjunction with other therapeutics. Suitable optional ingredients or other therapeutics include those conventional for treating conditions of this nature, e.g. other anti-inflammatories, diuretics, and immune suppressants, among others. Desirably, these modified chemokines are particularly well suited for administration in conjunction with colony stimulating factor.

IV. Methods for Mobilizing Hematopoietic Stem Cells

The invention provides improved methods of treating conditions characterized by immunosuppression or low numbers of hematopoietic stem cells and cells differentiated therefrom, including, without limitation, inflammation, fev r, viral, fungal, and bacterial

infections, cancer, myelopoietic dysfunction, hematopoiesis disorders, aplastic anemia, and autoimmune diseases, and conditions characterized by low production and/or differentiation of hematopoietic and/or bone marrow cells. This method involves administering to a selected mammal a pharmaceutical composition of the invention. Preferably, this composition is administered together with, or contains, a colony stimulating factor. Suitable sources of colony stimulating factor are well known and include, e.g., natural, synthetic and recombinant GM-CSF, M-CSF, G-CSF and IL-3. In another preferred embodiment, a desamino chemokine useful in the invention can be administered *in vivo*, and permitted to act in synergy with the natural colony stimulating factors found in a selected patient.

In one preferred embodiment, the method of the invention uses the desamino chemokines described herein in conjunction with GM-CSF (or G-CSF). The use of a modified chemokine, such as a desamino *groβ*, according to the method of the invention in combination with CSF (this combination has been observed to have synergy) permits lower doses of CSF to be administered to a patient, reducing the extremely unpleasant side effects caused by GM-CSF (G-CSF).

The mature chemokines and the modified or multimeric chemokines useful in the method of the invention are characterized by the ability to mobilize hematopoietic stem cells when administered alone, or by having synergistic activity in stimulating hematopoiesis when administered <u>in vivo</u> and <u>in vitro</u> with another hematopoietic factor, such as a colony stimulating factor or a growth factor, or combined with naturally circulating CSFs, or administered in a protocol with chemotherapy.

In one embodiment, the invention provides a method for mobilizing hematopoietic stem cells in an animal by administering to an animal an effective amount of the composition or medicament containing a mature chemokine selected from human *groβ* [SEQ ID NO: 3], human *groα* [SEQ ID NO:2], human *groγ* [SEQ ID NO:4], and murine KC [SEQ ID NO:1].

In another, embodiment of this invention, a method for mobilizing hematopoietic stem cells in an animal involves administering to an animal an effective amount of a modified protein derived from a chemokine selected from *groβ*, *groα*, *groγ*, and KC. As preferred embodiment there is provided a method for mobilizing hematopoietic stem cells in an animal by administering to an animal an effective amount of a modified protein derived from chemokine human *groβ* [SEQ ID NO: 3].

In still another aspect, the present invention provides a method for mobilizing hematopoietic stem cells in an animal comprising administering to an animal an effective amount of a multimeric protein, which comprises an associated of at least one chemokine as described above and a second chemokine.

In the practice of the method of mobilizing hematopoietic stem cells, the term "effective amount" of these proteins may be defined as that amount which, when administered to a patient by suitable means, mobilizes hematopoietic stem cells and increases the number of hematopoietic stem cells in the peripheral blood. This amount is expected to be higher than the amount required to stimulate the growth or development of hematopoietic progenitor cells. The effective amount increases in the circulation the cells which are differentiated from the hematopoietic stem cells in applicable clinical or veterinary situations. A desirabl effective amount may be about 0.01 ng/kg to 10 mg/kg body weight per dose.

Suitable means of administration for mobilizing stem cells include, without limitation, bolus injection or incremental administration of the effective amount by injection, i.v. drip, or any other appropriate internal route. Dosages may be repeated as needed, e.g. one to three times daily for between 1 day to about one week.

Additionally, the method of this invention employing the mature chemokines, or modified or multimeric chemokines identified above may be used in peripheral blood hematopoietic stem cell transplantation regimens. For example, following an optional initial dose of a chemotherapeutic agent, the mature chemokines or modified or multimeric chemokines identified above are administered in place of the CSFs now used to mobilize hematopoietic stem cells from the bone marrow to the peripheral circulation for harvesting, as well as for readministration following high doses of chemotherapy. Suitable chemotherapy agents include, without limitation, the well-known agents such as cyclophosphamide, cisplatinum, ARA-C, 5-fluorouracil, etopside, epirubicin, carboplatin, busulfan, mitoxantrone and carmustine. When administered with the chemokines according to this invention, the amounts of the chemotherapeutics are those amounts conventionally employed, i.e., about 1.2g/m^2 etopside, 800 μg/m^2 ARA-C, 200 mg/kg cyclophosphamide, etc. See for such dosages Hass *et al*, Seminars in Oncol., 21:19-24 (1994), incorporated herein by reference.

The chemokines identified above may be used to complement the conventionally used CSFs in treatment regimens. Alternatively, the chemokines identified above may be used in combination therapies with other hematopoietic regulatory biomolecules, such as the molecules involved in hematopoiesis above-referenced, or

with growth factors, conventional pharmaceuticals and/or drugs, for the same purposes. Suitable sources of such growth factors are well known and include, without limitation, natural, synthetic and recombinant GM-CSF, G-CSF, stem cell factor, and Flt-3 ligand. Other suitable biomolecules include (S)-5-oxo-L-prolyl-α-glutamyl-L-α-aspartyl-N^8-(5-amino-1-carboxypentyl)-8-oxo-N^7-[N-{N-(5-oxo-L-prolyl)-L-α-glutamuyl}-L-α-aspartyl]-L-threo-2,7,8-triaminooctanoyl-lysine [$(pGlu\text{-}Glu\text{-}Asp)_2$-Sub-$(Lys)_2$] [Pelus et al, Exp. Hematol., 22:239-247 (1994)]. Still other pharmaceuticals and drugs for co-administration may be readily selected by one of skill in the art.

The advantages of the use of this invention in replacement or in conjunction with traditional methods of peripheral blood hematopoietic stem cell transplantation are that more rapid recovery of PMNs and/or platelets occur than with bone marrow transplantation, the risk of infection is reduced and the method permits potentially higher curative doses of chemotherapy, or a series of dose intensified chemotherapy to be administered.

The following examples are illustrative only and do not limit the scope of the invention.

Example 1 - Mobilization Assay

Chemokines derived from KC [SEQ ID NO:1, *groß* [SEQ ID NO:3] and *groγ* [SEQ ID NO:4] including modified and multimeric chemokines are prepared using known techniques. See, e.g., WO94/29341 for additional discussion relating to the preparation of such chemokines. These chemokines are tested for the ability to mobilize hematopoietic stem cells in mice. Each chemokine is assayed in concentrations of 50, 10 and 2 μg/mouse and administered via subcutaneous,

intramuscular, intraperitoneal, intravenous, or oral route. The kinetics of chemokine mobilization of hematopoietic stem cells are monitored in 15 minute intervals over a period of 60 minutes by collecting blood samples by cardiac puncture from the mice. The mobilized hematopoietic stem cells are fractionated and collected by separation over a Lympholyte-M™ density gradient. Cells are washed for future use.

Mature blood cell elements are enumerated using a Technicon™ H1 hematology analyzer, equipped with veterinary software. Mobilization of mature inflammatory cells, such as polymorphonuclear (PMN) cells, eosinophils, and basophils are taken in to account when evaluating the overall potential inflammatory profile.

To monitor early and later hematopoietic progenitor cells, a CFU-GM assay is performed, i.e. blood samples collected during the mobilization phase are assessed for colony forming units (CFU-GM) at days 7 and 14. Cells are adjusted to 10^6 cells/ml in McCoys medium without serum. A single layer agar system consisting of McCoys medium enriched with nutrients ($NaHCO_3$, pyruvate, amino acids, vitamins and HEPES buffer), 0.3% Bacto agar, and 15% fetal bovine serum is used. Cells from the blood samples (final concentration of 10^5 cells/ml) are added to the agar system. The agar plates are incubated at 37°C, 7.5% CO_2 for 7-14 days. Colonies of proliferating cells (CFU-GM) are counted utilizing a microscope.

In addition, early hematopoietic high proliferative potential (HPP) progenitors, are counted in the day 14 CFU cultures.

The chemokine IL-8, which mobilizes hematopoietic stem cells as a single factor, is included in these studies as a positive control.

Preliminary experim nts have shown that administration of *groß* [SEQ ID NO: 3] results in a dose dependent mobilization of CFU-GM, similar to the results with the control. Modified *groß*, the N-terminal 4 amino acid truncation protein (aa5-73) of *groß* mobilized significantly greater numbers of hematopoietic progenitor cells than *groß* (amino acids 1-73) or IL-8. No significant changes (> 3 fold) in mature cell elements were observed in *groß* treated mice, indicating specific mobilization of hematopoietic progenitor cells. This result demonstrates that the modified desamino chemokines may have enhanced mobilization characteristics compared to the mature proteins.

Example 2 - Mobilization Assay in Combination with Hematostimulants

Hematostimulants are assayed in combination with the chemokines identified above as mobilization factors. The hematostimulants include G-CSF, GM-CSF, (S)-5-oxo-L-prolyl-α-glutamyl-L-α-aspartyl-N^8-(5-amino-1-carboxypentyl)-8-oxo-N^7-[N-{N-(5-oxo-L-prolyl)-L-α-glutamuyl}-L-α-aspartyl]-L-threo-2,7,8-triaminooctanoyl-lysine [$(pGlu\text{-}Glu\text{-}Asp)_2$-Sub-$(Lys)_2$] [Pelus, cited above] and FLT-3 ligand. Any G-CSF mimetic, i.e., a hematostimulant which is not a CSF like G-CSF or GM-CSF, but has hematopoietic activity, may be used.

In combination assays, the hematostimulant, e.g., G-CSF, is administered at 50 μg/kg to mice four days prior to the chemokines or modified or multimeric chemokines derived from KC [SEQ ID NO:1], *groß* [SEQ ID NO:3] and *groγ* [SEQ ID NO:4]. As in Example 1, the dose of chemokine and time of blood collection is varied.

A CFU-GM assay is performed as described above in Example 1, with SCF, IL-1 and GM-CSF as the source of colony stimulating activity. Mature blood cell elements, early and later progenitors are measured as for Example 1.

Combination studies with hematostimulant pre-treatment utilizes MIP-1α as the positive control.

Example 3 - Murine Peripheral Blood Stem Cell Transplantation Model

A. Mobilization of Primitive Long Term Repopulating Stem Cells

The following experiment was performed in an *in vivo* stem cell transplantation model to determine if N-terminally truncated *groβ* [aa 5-73 of SEQ ID NO:3; termed *groβ*-T] mobilizes primitive long term repopulating stem cells. In this model, gamma irradiated mice are recipients of bone marrow cells. Mice are followed for 100 days for survival.

The ability of blood stem cells collected from mice treated with either PBS, *groβ*-T (50 μg at 15 - 30 min.), G-CSF (1 μg/mouse BID x 4), or G-CSF, then *groβ*-T to rescue otherwise lethally irradiated mice. Blood mononuclear cells (up to 1E+6) collected from PBS treated mice protein 0-10% of the mice 100 days post transplant. Mice receiving marrow cells as the assay positive control were at 100% survival as of day 100. Mobilized blood cells (1E+6 cells/mouse) collected from mice treated with *groβ*-T alone protected 70% of recipients. Mobilized blood cells (1E+6 cells/mouse) collected from G-CSF treated donors protected 80% of recipients. Mobilized blood cells collected from donors treated with G-CSF and *groβ*-T mobilize greater numbers of repopulating cells than G-CSF alone.

B. Mobilization of Peripheral Blood Stem Cells

The rate at which peripheral blood stem cells mobilized by *groβ*-T recovered mature blood cell lineages in an irradiated host was evaluated. 1E+6 low density peripheral blood cells (LDPBC) were injected into irradiated recipients and bled by cardiac puncture on days 7-19 post irradiation. LDPBC from the different groups were collected under optimal conditions for CFU-GM mobilization. The groups compared in this experiment were PBS, *groβ*-T alone (50 μg,, 15 min), G-CSF (BID x 5 days, 1 μg/mouse alone), and *groβ*-T + G-CSF. Normal mice were bled daily for comparison to the transplanted animals.

Mice which received a transplant from PBS treated donors failed to recover mature blood cell elements and died. The rate of neutrophil recovery in the mice which received cells mobilized by truncated *groβ* was faster than those who received G-CSF mobilized cells. Mice transplanted with LDPBC mobilized by the combination of *groβ*-T + G-CSF resulted in a faster neutrophil recovery rate than *groβ*-T mobilized cells.

The recovery of platelet counts in these same mice followed the same pattern: *groβ*-T + G-CSF > *groβ*-T > G-CSF > > PBS. However, on day 19, platelet counts are still far from returning to normal values. These data indicate that *groβ*-T mobilized blood stem cells engraft in recipient mice, with resultant neutrophil and platelet recovery rates equal to or better than G-CSF mobilized stem cells.

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: Method of Mobilizing Hematopoietic Stem Cells

(iii) NUMBER OF SEQUENCES: 4

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: WO
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/547,262
(B) FILING DATE: 24-OCT-1995

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(C) REFERENCE/DOCKET NUMBER: SBC P50161-2PCT

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Ala Pro Ile Ala Asn Glu Leu Arg Cys Gln Cys Leu Gln Thr Met
 1               5                  10                  15

Ala Gly Ile His Leu Lys Asn Ile Gln Ser Leu Lys Val Leu Pro
            20                  25                  30

Ser Gly Pro His Cys Thr Gln Thr Glu Val Ile Ala Thr Leu Lys
            35                  40                  45

Asn Gly Arg Glu Ala Cys Leu Asp Pro Glu Ala Pro Leu Val Gln
            50                  55                  60

Lys Ile Val Gln Lys Met Leu Lys Gly Val Pro Lys
            65                  70
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Ala Ser Val Ala Thr Glu Leu Arg Cys Gln Cys Leu Gln Thr Leu
 1               5                  10                  15

Gln Gly Ile His Pro Lys Asn Ile Gln Ser Val Asn Val Lys Ser
            20                  25                  30

Pro Gly Pro His Cys Ala Gln Thr Glu Val Ile Ala Thr Leu Lys
            35                  40                  45

Asn Gly Arg Lys Ala Cys Leu Asn Pro Ala Ser Pro Ile Val Lys
            50                  55                  60

Lys Ile Ile Glu Lys Met Leu Asn Ser Asp Lys Ser Asn
            65                  70
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ala Pro Leu Ala Thr Glu Leu Arg Cys Gln Cys Leu Gln Thr Leu
1 5 10 15

Gln Gly Ile His Leu Lys Asn Ile Gln Ser Val Lys Val Lys Ser
20 25 30

Pro Gly Pro His Cys Ala Gln Thr Glu Val Ile Ala Thr Leu Lys
35 40 45

Asn Gly Gln Lys Ala Cys Leu Asn Pro Ala Ser Pro Met Val Lys
50 55 60

Lys Ile Ile Glu Lys Met Leu Lys Asn Gly Lys Ser Asn
65 70

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ala Ser Val Val Thr Glu Leu Arg Cys Gln Cys Leu Gln Thr Leu
1 5 10 15

Gln Gly Ile His Leu Lys Asn Ile Gln Ser Val Asn Val Arg Ser
20 25 30

Pro Gly Pro His Cys Ala Gln Thr Glu Val Ile Ala Thr Leu Lys
35 40 45

Asn Gly Lys Lys Ala Cys Leu Asn Pro Ala Ser Pro Met Val Gln
50 55 60

Lys Ile Ile Glu Lys Ile Leu Asn Lys Gly Ser Thr Asn
65 70

WHAT IS CLAIMED IS:

1. Use of a protein derived from a mammalian chemokine selected from the group consisting of (a) *groα* SEQ ID NO:2, (b) *groß* SEQ ID NO: 3, (c) *groγ* SEQ ID NO:4; and (d) KC SEQ ID NO:1, in the preparation of a medicament useful for mobilizing hematopoietic stem cells.

2. Use according to claim 1 wherein said chemokine is selected from the group consisting of:

(a) mature *groβ*;

(b) amino acids 5 to 73 of SEQ ID NO: 3;

(c) a multimeric chemokine protein which comprises an association of two or more of (a) or (b); and

(d) a multimeric chemokine protein which comprises an association of (a) or (b) with a second chemokine.

3. Use according to claim 1 wherein said chemokine is selected from the group consisting of:

(a) mature *groα*;

(b) amino acids 5 to 73 of SEQ ID NO: 2;

(c) a multimeric chemokine protein which comprises an association of two or more of (a) or (b); and

(d) a multimeric chemokine protein which comprises an association of (a) or (b) with a second chemokine.

4. Use according to claim 1 wherein said chemokine is selected from the group consisting of:

(a) mature *groγ*;

(b) amino acids 5 to 73 of SEQ ID NO: 4;

(c) a multimeric chemokine protein which comprises an association of two or more of (a) or (b); and

(d) a multimeric chemokine protein which comprises an association of (a) or (b) with a second chemokine.

5. Use according to claim 1 wherein said chemokine is selected from the group consisting of:

(a) mature KC;

(b) amino acids 5 to 72 of SEQ ID NO: 1;

(c) a multimeric chemokine protein which comprises an association of two or more of (a) or (b); and

(d) a multimeric chemokine protein which comprises an association of (a) or (b) with a second chemokine.

6. Use according to claims 1-5 wherein said chemokine comprises between about 0.01 ng to about 1 g of said medicament.

7. Use according to claims 1-5, wherein said medicament is co-administered with a growth factor or other hematopoietic regulatory biomolecule.

8. Use according to claim 7 wherein said growth factor is selected from the group consisting of GM-CSF, G-CSF, stem cell factor, and Flt-3 ligand.

9. Use according to claim 7 where said biomolecule is (S)-5-oxo-L-prolyl-α-glutamyl-L-α-aspartyl-N^8-(5-amino-1-carboxypentyl)-8-oxo-N^7-[N-{N-(5-oxo-L-prolyl)-L-α-glutamuyl}-L-α-asparty1]-L-threo-2,7,8-triaminooctanoyl-lysine [$(pGlu\text{-}Glu\text{-}Asp)_2$-Sub-$(Lys)_2$].

10. Use according to claim 1 wherein the medicament is used in the treatment of a patient receiving peripheral blood hematopoietic stem cell transplantation.

11. Use according to claim 10 wherein the patient has received a dose of a selected chemotherapeutic agent prior to treatment with the medicament; the hematopoietic stem cells from the peripheral blood of the patient treated are harvested; a chemotherapeutic agent is administered; and the patient is reinfused with the harvested cells.

12. Use according to claim 11 wherein said chemotherapeutic agent is selected from the group consisting of cyclophosphamide, cisplatinum, ARA-C, 5-fluorouracil, etopside, epirubicin, carboplatin, busulphan, mitoxantrone and carmustine.

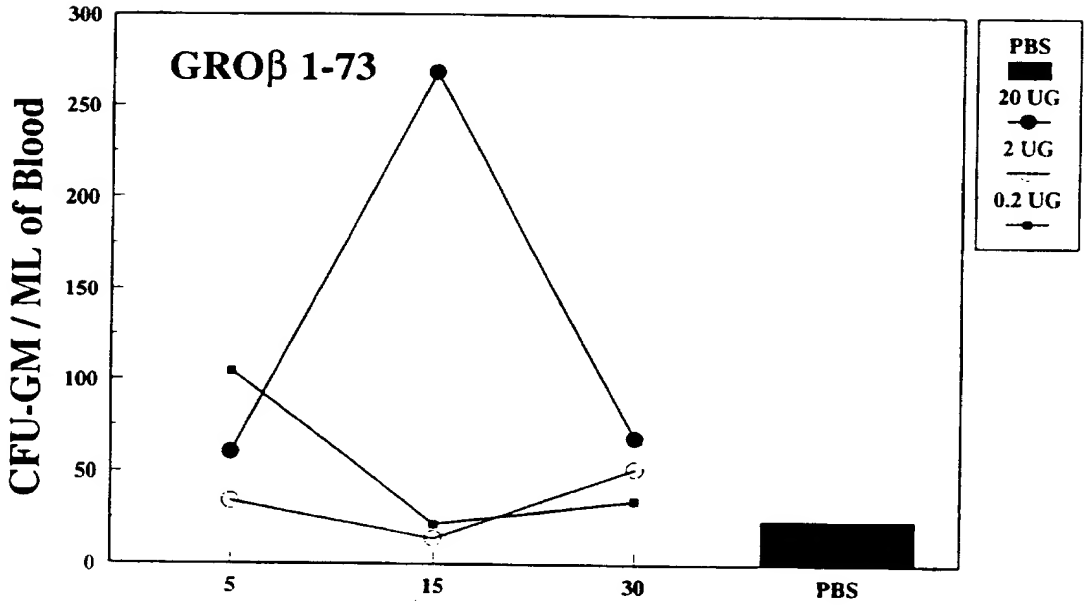

Figure 1

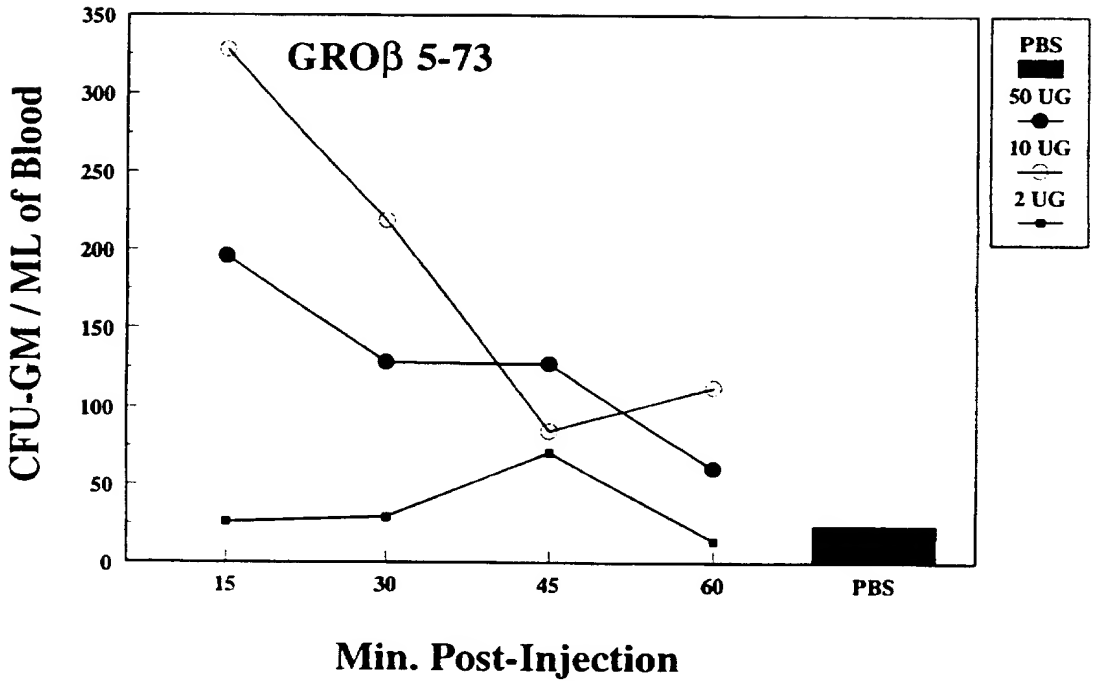

Figure 2

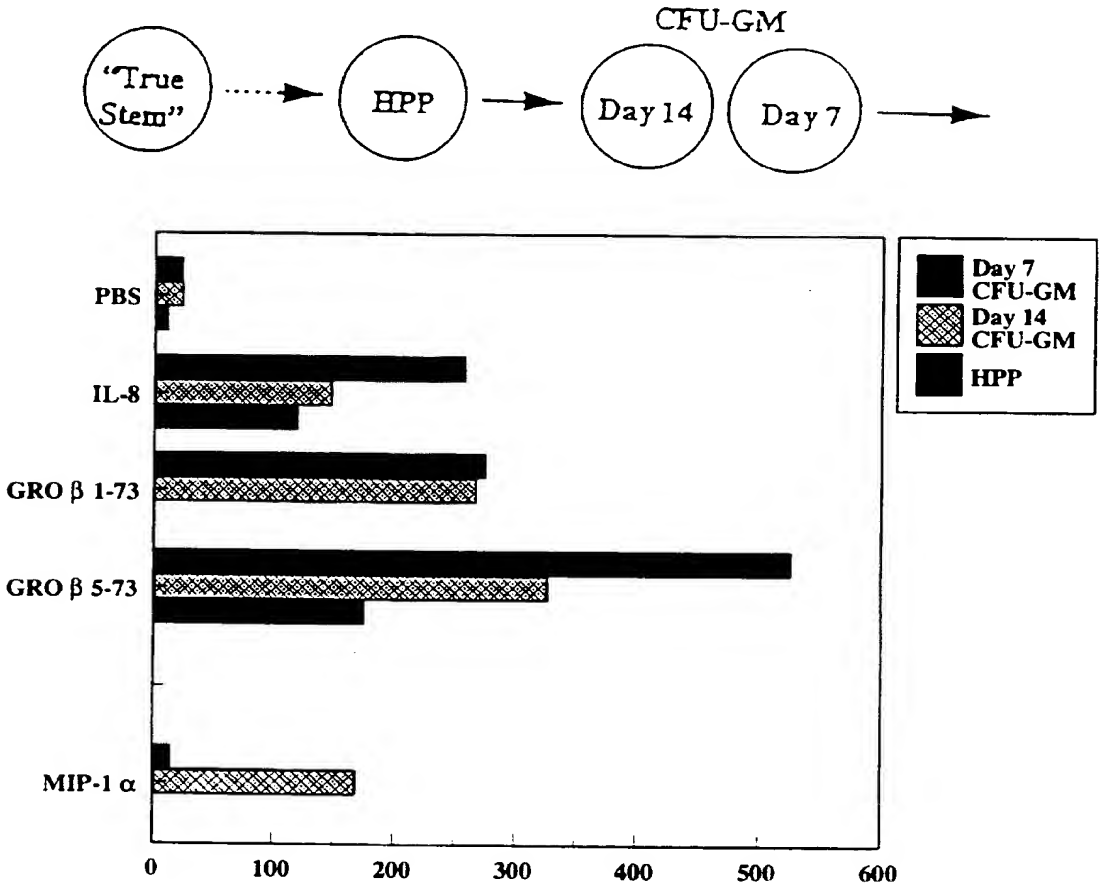

Figure 3

INTERNATIONAL SEARCH REPORT

International application N .
PCT/US96/17074

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/52, 14/47; A61K 38/16, 38/17, 38/19; C12N 15/19
US CL :424/85.1; 435/69.5; 514/2,8,12,885; 530/351
According to International Patent Classification (IPC) r to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.1; 435/69.5; 514/2,8,12,885; 530/351

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	CAO et al. gro-beta, a -C-X-C- Chemokine, Is an Angiogenesis Inhibitor That Suppresses the Growth of Lewis Lung Carcinoma in Mice. J. Exp. Med. December 1995, Vol.182, pages 2069-2077.	1-12
A	CUENCA et al. Characterization of GRO alpha, beta and gamma expression in human colonic tumours: potential significance of cytokine involvement. Surgical Oncology. 1992, Vol.1, pages 323-329.	1-12
A	BECKER et al. Constitutive and stimulated MCP-1, GROalpha, beta, and gamma expression in human airway epithelium and bronchoalveolar macrophages. American Journal of Physiology. March 1994, Vol.266, pages L278-288.	1-12

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 22 JANUARY 1997	Date of mailing of the international search report 25 FEB 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PREMA MERTZ Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application N .
PCT/US96/17074

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, f the relevant passages	Relevant to claim No.
A	STOECKLE, M.Y. Post-transcriptional regulation of groalpha, beta, gamma, and IL-8 mRNAs by IL-1beta. Nucleic Acids Research. 25 February 1991, Vol.19, No.4, pages 917-920.	1-12

INTERNATIONAL SEARCH REPORT

International application N .
PCT/US96/17074

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS ONLINE, MEDLINE, BIOSIS
search terms:chemokine, gro-beta or macrophage inflammatory protein-2 alpha, gro-alpha, gro-gamma or macrophage inflammatory protein-2 beta, mouse macrophage inflammatory protein-2 or KC, administration or treatment or therapy.